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FUSION OF ERYTHROCYTES BY NEWCASTLE DISEASE VIRUS

E. TRYBALA

Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Academy of Agriculture and Technology in Olsztyn, Kortowo II, bl. 105E, 10—957 Olsztyn, Poland

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Summary. — Newcastle disease virus-induced fusion of chick embryo (CE) and chicken erythrocytes has been studied at the ph range between 5.5 and 8.0. The highest degree of fusion of CE erythrocytes was observed at pH 5.5, whereas the chicken erythrocytes fused at pH 5.5—6.0 only. Freezing and thawing of low-haemolytic virus preparation increased its erythrocyte fusion activity. Ammonium chloride did not cause a statistically significant effect on the multiplication of virus preparations expressing different haemolysis and erythrocyte fusion activity.

Key words: Newcastle disease virus; fusion of erythrocytes; pH dependence

The phenomenon of virus-induced fusion of erythrocytes has been characterized in detail for Sendai virus (Bächi *et al.*, 1973; Knutton *et al.*, 1977; Knutton, 1977; 1978; 1979; 1980; Knutton and Bächi, 1980) and in limited extent for the second important paramyxovirus, the Newcastle disease virus (NDV) (Terry and Ho-Terry, 1976; Knutton *et al.*, 1977). Therefore, in the present paper attention has been paid to the conditions necessary for induction of erythrocyte fusion by the latter virus.

The lentogenic LaSota strain of NDV was used. The virus was inoculated into the allantoic cavity of 10-day-old CE at an input multiplicity of ten 50 % egg infectious doses (EID_{50}) per cell, calculated on the basis of data provided by Cuadra (1975), according which the number of cells lining the allantoic cavity is about 4.1×10^7. Infectious allantoic fluid was harvested after 24 hr (early harvest) or after 96 hr (late harvest), incubation at 37.8 °C. Virus preparations were used inducing a haemolysis at pH 7.0 to the following extent: a) 0—3 % — infectious allantoic fluid originating from the selected early egg harvest (EH); b) 20—25 % — infectious allantoic fluid originating from selected eggs of late harvest (LH); c) 50—55 % — early harvest of the virus which had been subjected to ten cycles of freezing and thawing (EH-10). Chicken (hen) and 13—14-day-old chick embryo erythrocytes were used throughout. The latter were obtained by bleeding the CE into the allantoic cavity and triple washing with PBS-A (without calcium and magnesium ions). Erythrocyte fusion test was carried out as described by Kitame *et al.* (1982); equal volumes of the infectious allantoic fluid (254 HAU) and 0.025 % chicken or chick embryo erythrocytes were mixed. After adsorption (45 min at 4 °C), red blood cells were sedimented at $250 \times g$ for 5 min and resuspended in 0.2 ml of isotonic 0.02 mol/l phosphate buffer (pH 6.0—8.0) or in an isotonic 0.02 mol l acetate buffer (pH 5.5). Fusion was stopped by the addition of 50 µl of 5 % glutaraldehyde in the same buffer and observed under the microscope at magnification of ×200. As there are no satisfactory instrumental methods for measuring erythrocyte fusion, its degree was expressed as described by Väänänen and Kääriäinen (1980).

Table 1. pH dependence of chicken and CE erythrocyte fusion induced by EH, LH and EH-10 LaSota strain preparations

Virus preparation	Red blood cells	pH			
		5.5	6.0	7.0	8.0
EH	ChE	++	−	−	−
	CEE	+++	++	+	+
LH	ChE	+++	−	−	−
	CEE	+++	++	+	++
EH-10	ChE	n.t.	++	−	−
	CEE	++++	+++	++	+++

Note: n.t. = not tested; ChE = chicken erytrocytes; CEE = chick embryo erythrocytes; − = no fusion; +, ++, +++ and ++++ = intensity of fusion; EH, LH and EH-10 − for explanations see text.

As shown in Table 1, EH, LH and EH-10 LaSota strain preparations caused the highest fusion of chick embryo erythrocytes at pH 5.5, whereas chicken erythrocytes were fused only at pH 5.5—6.0 Fusion of CE erythrocytes induced by EH-10 preparation at pH 5.5 is presented in Fig. 1. NDV--induced fusion of erythrocytes has so far been studied only at neutral pH (Terry and Ho-Terry, 1976; Knutton *et al.*, 1977). In this paper, no fusion of chicken erythrocytes and only minimal fusion of CE erythrocytes were

Table 2. pH dependence of haemolysis induced by EH, LH and EH-10 LaSota strain preparations

Virus preparation	Red blood cells	pH				
		5.5	6.0	6.5	7.0	8.0
	ChE	3.8 (5.7)	4.2	2.6	2.3	0.7
EH	CEE	2.0 (4.5)	2.0	1.1	0.8	0.7
	ChE	30.6 (50.6)	31.2	27.6	24.0	15.1
LH	CEE	22.2 (40.7)	18.5	13.8	10.7	15.5
	ChE	80.7 (76.8)	81.2	73.5	57.7	60.8
EH-10	CEE	72.3 (52.4)	47.5	46.1	44.4	57.8

Note: Results expressed in per cent of haemolysis; in parentheses haemolytic activity measured in an acetate buffered saline. For further explanations see Table 1.

Table 3. Effect of ammonium chloride on the replication of EH, LH and EH-10 LaSota strain preparations

	EH	LH	EH-10
Ammonium chloride	$10^{4.69a}$	$10^{5.37}$	$10^{4.19}$
Control	$10^{5.19}$	$10^{5.43}$	$10^{4.25}$

[a] Reciprocal of the ID_{50} titre
For further explanations see text.

observed at pH 7.0. Similar results were also obtained with other strains of NDV — B_1 and Roakin (data not shown).

Because cell fusion and haemolytic properties of paramyxoviruses are tighly coupled phenomena (Scheid and Choppin, 1974; Knutton and Bächi, 1980), the pH dependence of haemolysis by EH, LH and EH-10 preparations were examined next. Determinations were carried out as described by Shibata *et al.* (1982), but after adsorption and sedimentation, erythrocytes were washed with cold PBS-A and resuspended in 0.02 mol/l phosphate--buffered saline at pH 5.5—8.0 or in an acetate-buffered saline at pH 5.5. At this pH range no spontaneous haemolysis occured. As shown in Tables 1 and 2, both erythrocyte fusion and haemolysis revealed the highest levels at low pH. Both these properties were also increased by freezing and thawing of low-haemolytic EH virus preparation. Nevertheless, some discrepancies between these phenomena were observed. Namely, despite higher level of haemolytic activity of LH in comparison to EH preparation, there were no marked differences between their CE erythrocyte fusion properties.

The ability of NDV to induce extensive fusion of erythrocytes at low pH can be explained either by its weaker elution at this pH (Sagik and Levine, 1957) or by the effect of low pH upon the virus envelope. However, other possibilities cannot be excluded.

The results of our studies also suggest that unlike Sendai virus which causes erythrocyte fusion at pH 6.6 and above (Väänänen and Kääriäinen, 1980), NDV seems to be somewhat similar to orthomyxo-, rhabdo- and togaviruses which induce fusion at rather low pH (Maeda and Ohnishi, 1980; Väänänen and Kääriäinen, 1980; Mifune *et al.*, 1982). It has been established, that infectious cell entry mechanism of viruses which cause cell fusion and haemolysis only at low pH is based on virus uptake by endocytosis (Helenius *et al.*, 1980; Matlin *et al.*, 1982; Yoshimura *et al.*, 1982). Their multiplication can be inhibited in this stage by lipophilic weak bases such as ammonium chloride, chloroquine and amantadine (Miller and Lenard, 1980; Helenius *et al.*, 1982; Yoshimura *et al.*, 1982). Hence, considering the observed similarities, it was of interest to know whether the EH, LH and EH-10 of

LaSota strain replication were blocked by ammonium chloride. Determinations were carried out in CE cells as described by Yoshimura *et al.* (1982). Infectious titres were calculated according to Reed and Muench. The statistical significance of differences was established from Lorenz's tables (1960). Because no statistically significant differences were observed (Table 3), it can be assumed, that NDV invasion of cells, irrespective of the level of its haemolytic and erythrocyte fusion activities, may not require a low pH--mediated step. These data are in agreement with the studies by Nagai *et al.* (1983). It has been observed earlier that both non-haemolytic as well as haemolytic harvests of Sendai virus enter cells by direct fusion of the virus envelope with the cell membrane (Shimizu *et al.*, 1976; Knutton, 1977; 1979).

In the next paper the use of the erythrocyte fusion in elaboration of haemofusion inhibition test for assessment of antibody response against NDV will be described (Trybala, manuscript in preparation).

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References

Bächi, T., Aguet, M., and Howe C. (1973): Fusion of erythrocytes by Sendai virus studied by immuno-freeze-etching. *J. Virol.* **11**, 1004—1012.

Cuadra, M. (1975): The growth cycle of influenza viruses as studied in touch preparations of the allantoic layer of chick embryos. I. The number of cells lining allantoic cavity. *Zbl. Bakt. ParasitKde Abt. I. A* **231**, 239—420.

Helenius, A., Kartenbeck, J., Simons, K., and Fries, F. (1980): On the entry of Semliki Forest virus into BHK-21 cells. *J. Cell Biol.* **84**, 404—420.

Helenius, A., Marsh, and White, J. (1982): Inhibition of Semliki Forest virus penetration by lysosomotropic weak bases. *J. gen. Virol.* **58**, 47—61.

Kitame, F., Sugawara, K., Ohwada, K., and Homma, M. (1982): Proteolytic activation of hemolysis and fusion by influenza C virus. *Arch. Virol.* **73**, 357—361.

Knutton, S. (1977): Studies of memrane fusion. II. Fusion of human erythrocytes by Sendai virus. *J. Cell Sci.* **28**, 198—210.

Knutton, S. (1978): The mechanism of virus-induced cell fusion. *Micron* **9**, 133—154.

Knutton, S. (1979): Studies of membrane fusion. V. Fusion of erythrocytes with non-hemolytic Sendai virus. *J. Cell Sci.* **36**, 85—96.

Knutton, S. (1980): Studies of membrane fusion. VI. Mechanism of the membrane fusion and cell swelling stages of Sendai virus-mediated cell fusion. *J. Cell Sci.* **43**, 103—118.

Knutton, S., and Bächi, T. (1980): The role of cell swelling and haemolysis in Sendai virus--induced cell fusion and in the diffusion of incorporated viral antigens. *J. Cell Sci.* **42**, 153—167.

Knutton, S., Jackson, D., and Ford, M. (1977): Studies of membrane fusion. I. Paramyxovirus--induced cell fusion, a scanning electron-microscope study. *J. Cell Sci.* **28**, 179—188.

Lorenz, R. J. (1960): Untersuchungen über die Dosis-Wirkungs-Beziehung und die Genauigkeit bei Virustitrierungen. *Arch. ges. Virusforsch.* **10**, 560—568.

Maeda, T., and Ohnishi, S. (1980): Activation of influenza virus by acidic media causes hemolysis and fusion of erythrocytes. *FEBS Letters* **122**, 283—287.

Matlin, K. S., Reggio, H., Helenius, A., and Simons, K. (1982): Pathway of vesicular stomatitis virus entry leading to infection. *J. molec. Biol.* **156**, 609—631.

Mifune, K., Ohuchi, M., and Mannen, K. (1982): Hemolysis and cell fusion by rhabdoviruses. *FEBS Letters* **137**, 293—297.

Miller, D. K., and Lenard, J. (1980): Inhibition of vesicular stomatitis virus infection by spike glycoprotein. *J. Cell Biol.* **84**, 430—437.

Nagai, Y., Hamaguchi, M., Toyoda, T., and Yoshida, T. (1983): The uncoating of paramyxoviruses may not require a low pH mediated step. *Virology* **130**, 263—268.

Sagik, B. P., and Levine, S. (1957): The interaction of Newcastle disease virus (NDV) with chicken erythrocytes: attachment, elution and hemolysis. *Virology* **3**, 401—416.
Scheid, A., and Choppin, P. W. (1974): Identification of biological activities of paramyxovirus glycoproteins. Activation of cell fusion, hemolysis and infectivity by proteolytic cleavage of an inactive precursor protein of Sendai virus. *Virology* **57**, 475—490.
Shibata, M., Maeno, K., Tsurumi, T., Aoki, H., Nishiyama, Y., Ito, Y., Isomura, S., and Suzuki, S. (1982): Role of viral glycoproteins in haemolysis by influenza B virus. *J. gen. Virol.* **59**, 183—186.
Shimizu, Y. K., Shimizu, K., Ishida, N., and Homma, M. (1976): On the study of Sendai virus haemolysis. II. Morphological study of envelope fusion and haemolysis. *Virology* **71**, 48—60.
Terry, G. M., and Ho-Terry, L. (1976): Fusion and haemolysis of chick erythrocytes by Newcastle disease virus. *Arch. Virol.* **50**, 37—44.
Väänänen, P., and Kääriäinen, L. (1980): Fusion and haemolysis of erythrocytes caused by three togaviruses: Semliki Forest, Sindbis and rubella. *J. gen. Virol.* **46**, 467—475.
Yoshimura, A., Kuroda, K., Kawasaki, K., Yamashina, S., Maeda, T., and Ohnishi, S. (1982): Infectious cell entry mechanism of influenza virus. *J. Virol.* **43**, 284—293.

Explanation of Micrographs (Plate XXXVIII):

Fig. 1. — Newcastle disease virus-induced fusion of CE erythrocytes.
Fig. 1. - I — Fusion of erythrocytes by EH-10 LaSota strain at pH 5.5 (160×).
Fig. 1. - II — Control, CE erythrocytes (160×).